SHIBAYAGI	Bovine Albumin ELISA Kit	Research Reagent

Please, read this instruction carefully before assay.

[Advantage]

(1) Bovine albumin can be measured rapidly (Total reaction time: 2.5 hrs.).

(2) This Kit requires small volume of samples (100µl).

(3) This Kit can be used immediately because all reagents are provided in solutions.

(4) This Kit ensures excellent precision and reproducibility.

[Reagents]

A:	Anti-bovine albumin-coated plate	96well(8x12)	x1
B:	Standard bovine albumin solution (500ng/ml)	200µl	x1
C:	Buffer solution	60ml	x1
D:	Peroxidase-conjugated anti-albumin antibody	200µl	x1
F:	Chromogenic substrate reagent (TMB)	12ml	x1
H:	Reaction stopper (1M H_2SO_4)	12ml	x1
I:	Concentrated washing buffer (10x)	100ml	x1

[Assay samples]

Samples suspected to be contaminated with minute bovine albumin.
Samples should be properly diluted with assay buffer when assayed.

[Assay range]

0.78 – 50ng/ml

[Operation of the kit]

1. Equipments necessary but not included in the kit

(1) Micropipette
(2) Microplate washer or jet bottle.
(3) Microplate reader

2. Preparation of assay reagents

*Reagent solutions should be used immediately after preparation.
All the reagents of the kit should be used after getting back to room Temperature (20 – 25C).

(1) Washing buffer
Prepare by dilution of concentrated bffer (I) with purified water to 1:10.

(2) HRP-conjugated antibody
Prepare by dilution of (D) with the buffer(C) to 1:100.

(3) Other reagents should be used as they are without dilution.

3. Preparation of standard albumin solutions (an example)

Albumin conc. (ng/ml)	50	25	12.5	6.25	3.13	1.56	0.78	0
Standard albumin (μl)	50*	250**	250**	250**	250**	250**	250**	0
Buffer solution (μl)	450	250	250	250	250	250	250	250

4. Assay Procedure

Remove the cover seal of the microplate after getting back to room temperature.
If the cover seal is peeled off while it is still cool, it may be broken, and fragments may remain on the surface of the plate.

1) Rinse the anti-insulin coated plate 4 times with washing buffer.
Finally, hold the plate upside down, and strike the plate against folded several sheets of paper towel to remove washing buffer as completely as possible.

2) Pipette 100μl of assay sample or standard solution into corresponding wells, and shake.

3) Incubate for 1 hour at room temperature.(20-25C).

4) Wash the plate 4 times with washing buffer, and finally remove the buffer as is described in step 1.

5) Pipette 100μl of peroxidase-conjugated antibody solution into each well and shake.

6) Incubate for 1 hour at room temperature. (20-25C).

7) Wash the plate 4 times with washing buffer, and finally remove the buffer as is described in step 1.

8) Pipette 100μl of chromoqenic substrate solution into each well and shake.

9) Incubate for 30 minutes at room temperature (20-25C).

10) Pipette 100ml of Reaction stopper into each well and shake.

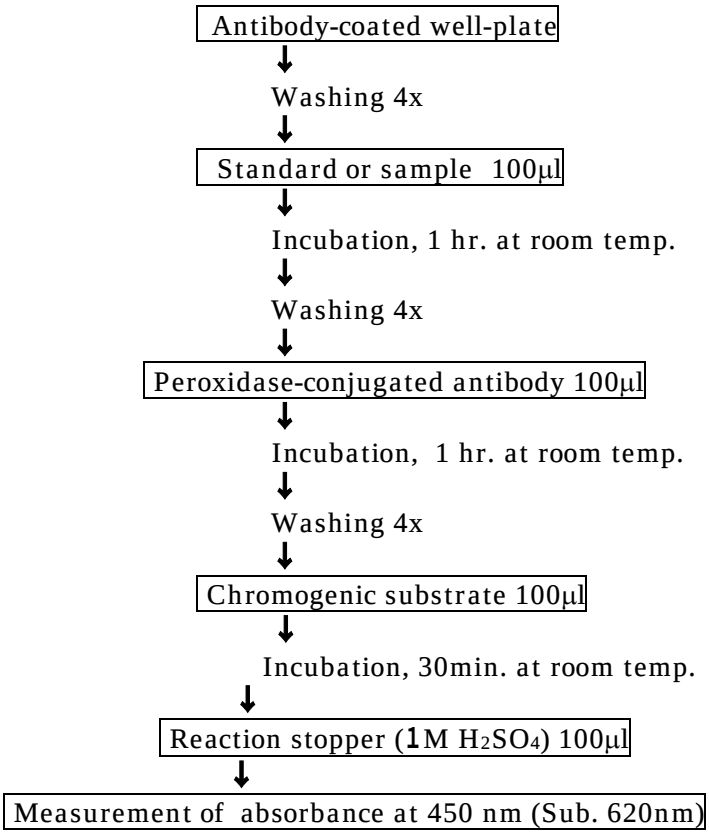
11) Measure each well's absorbance at 450 nm (sub-wave length 620nm) by a

plate reader within 30 minutes.

5. Calculation of Albumin Concentration

1. Using hemi-logarithmic section paper, prepare a standard curve by plotting absorbance(Y-axis) against logarithm of albumin concentration (X-axis, ng/ml).
2. Using the standard curve, read the albumin concentration of a sample from their absorbance.
3. In case an assay sample is diluted, then multiply the concentration by sample dilution rate to obtain the albumin concentration of the original sample.

6. Summary of assay procedure



Room temp.: 20-25C

[Important notes about samples]

1. Treatments of assay samples

(1) This assay kit is suitable to detect and measure minute amounts of bovine albumin contaminated in samples. Adjust pH of assay sample to be in a range of 6.5 – 8.0. This kit is too sensitive to measure albumin in bovine serum or plasma samples. In such case, serum/plasma must be diluted more than 1: 1,000,000.

(2). If a sample contains insoluble matter or looks turbid, make the sample clear by centrifugation or other means.

(3) Sample should be assayed as soon as possible after preparation.
(4) Please, avoid using NaF-containing blood sampling tube, because fluoride ion is a peroxidase inhibitor, and may reduce the coloration even after washing.

2. Storage of sample

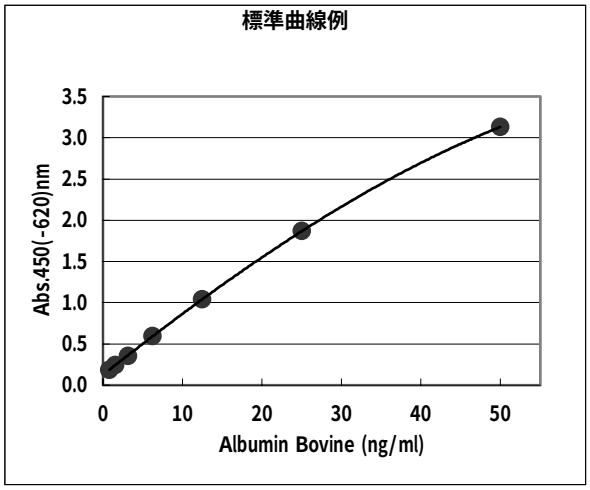
If samples have to be stored for some period, keep them frozen under –35C. Avoid from repeated freezing and thawing.

3. Influence of interfering substances

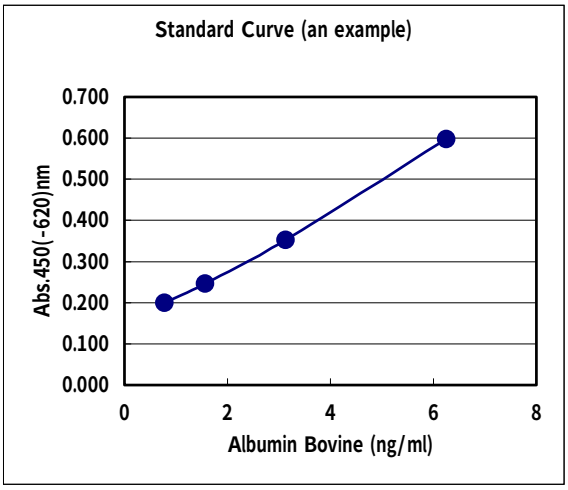
If presence of interfering substances is suspected in samples, assay them after 2 or more different dilution, and confirm the linearity of dilution-assay value.

[Standard curve and assay validation]

1. Standard curves (examples)



Total assay range



Lower concentration area

2. Specificity

Cross reaction to mouse, rat, and human albumin is less than lower assay limit.

3. Assay precision and reproducibility

(1) Within assay variation
Average C.V. is less than 5% (5 replicates assay, 4 samples)

(2) Between assay variation
Average C.V. is less than 5% (4 days, triplicates assay, 3 samples)

[Storage Condition]

2-8C, in a dark place,. Do not freeze.

[Valid period]

Six months after preparation. (valid limit is shown on the label of the container.

[Package unit]

96 wells /1 plate

[Product code]

AKRBS-018

[Statements and Precautions]

01. This assay kit or its components should be used only for research works.
02. The reagent solutions of the kit should be used principally immediately after dilution. Otherwise, keep them in a dark place at 2-8℃, and use them within 5 days.
03. The reagents were prepared to give accurate results by their combination within the kit. So, do not combine the reagents in the kit of other lot number. Even the lot number is the same, do not mix the reagents with those that are preserved for some period.
04. Pipetting and dilution of the reagent solutions should be made accurately because these steps influence the assay precision.
05. Do not dry the assay plate to avoid denaturation of the coated antibody or antigen.
06. The reaction time should be counted from the onset of reagent pipetting.
07. Prepare the standard curve in every assay.
08. Dilution of the assay sample must be carried out using the buffer solution attached to the kit.
09. Preservation condition for the kit or its components should be strictly kept.
10. ***Be careful not to allow the reagent solutions of the kit to contact with skin, mucus and eyes (wearing glasses for protection is recommended). Especially treat the stopping solution very carefully because it contains sulfuric acid.***
11. HRP-conjugated reagent solution, chromogenic substrate solution, and reaction stopper should be avoided from contacting with any metal.
12. ***In treating assay samples of animal origin, be careful for possible biohazards.***

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